

Pollen Morphological Studies on the Genus *Cirsium* Mill. (*Asteraceae*) in Iran

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Pollen morphology was studied in 23 Iranian species of *Cirsium* (*Asteraceae*) representing five sections, *Cirsium*, *Cephalonoplos*, *Echenais*, *Epitrachys* and *Pseudoepitrachys* by light and scanning electron microscopy. Among them five species are endemic to Iran and their pollen data are new to science. Pollen grains were radially symmetrical, isopolar, trizonocolporate and with echinate sculpturing. The tectum is microperforate, microreticulate or microgranulate in sculpture. The shape of pollen grains is suboblate to oblate-spheroidal in the equatorial view and is circular to triangular in polar view. The spines are conical with broad base and pointed or rounded tip. The number of spine rows between colpi varies from 4 to 5 among the species. ANOVA test performed for pollen characteristics showed no significant difference except for equatorial axis length, indicating that pollen data may not be used for delimitation or discrimination of those sections. PCA analysis showed the importance of echinae characteristics along with equatorial axis length, distance between two colpi and polar axis length in species delimitation. Clustering methods and the ordination plot of the PCA analysis support the affinity in the sect. *Pseudoepitrachys* and the distinction of the sect. *Cephalonoplos* from other sections, but did not support the grouping of the remaining sections from pollen morphology.

Key words: *Cirsium*, cluster analysis, Iran, PCA analysis, pollen morphology.

The genus *Cirsium* Mill., is one of the largest genera in the family *Asteraceae*, consisting of over 250 species of spiny perennial, biennial or rarely annual plants, and is distributed in the northern hemisphere, spanning the subtropical to boreal latitudes mainly distributed in Europe, North Africa, Asia, and North and Central America (Segarra-Moragues et al. 2007, Yildiz

and Dirmenci 2008). The highest species diversity is concentrated in the mountains of Southern Europe and in the Caucasus (Bures et al. 2004).

The genus is attributed to tribe *Cardueae* subtribe *Carduinae* in *Asteraceae* (Susanna et al. 2006). About 28 *Cirsium* species have been reported from Iran in the Flora Iranica

Table 1. *Cirsium* species examined in this study

Section/Species	Locality and voucher specimen
Sect. <i>Psudoepitrachys</i>	
<i>C. spectabile</i> DC.	Kerman: Ghgher (Baft), Ghonbadan, Mirtadzedini 32098 (TUH)
<i>C. congestum</i> Fitch. & C. A. Mey	Tehran : Shahrestanak, Khor village, 2500 m, Nouroozi & Fathollahi 8600155 (HSBU)
<i>C. pyramidale</i> Bornm.	Kerman: Baft, Hankuye, 2300 m, Mirtadzedini 23104 (TUH)
Sect. <i>Epitrachys</i>	
<i>C. bracteosum</i> DC.	Kerman: Ghogher, Cheshme sabz, Mirtadzedini 29488 (TUH)(Endemic)
<i>C. bornmulleri</i> Sint. ex Bornm	Khorasan: 47 km from Shirvan to Sarani, 1740 m, Ghahreman & Attar 27658 (TUH)
<i>C. strigosum</i> (M. Bieb.) M. Bieb.	Khorassan: Mashhad, 75 km to Kalat-e Naderi, Taherabad, 975 m, Ghahreman & Attar 27679 (TUH)
<i>C. ciliatum</i> (Murray) Moench	Azerbaijan: Salmas to Khoy, 1400 m, Nouroozi 8600160 (HSBU)
<i>C. osseticum</i> (Adams) Petrik	Azerbaijan: Arasbaran protected area, inter veighan and mahmoodabad, 1700 m, Ghahreman & Attar 17588 (TUH)
<i>C. aduncum</i> Fitch. & C. A. Mey	Azerbaijan: Meshkinshahr, Mazrae Jahan, 1169 m, Nouroozi & Fathollahi 8600170 (HSBU)
<i>C. haussknechtii</i> Boiss.	Azerbaijan: Tabriz, Kandovan, Hilevar village, 1600 m, Nouroozi 8600180 (HSBU)
<i>C. lappaceum</i> M. Bieb.	Azerbaijan: Meshkinshahr, Lahrood, Shabil, 2850 m, Ghahreman & Mozzafarian 70123 (TUH)
<i>C. turkestanicum</i> (Regel) Petrik	Khorasan: Between Shirvan to Sarani, zivar village, Ghahreman & Attar 27661 (TUH)
<i>C. vulgare</i> (Savi) Ten.	Tehran: Road of Karaj to Chaloos, Asara, 2050 m, Nouroozi 8600218 (HSBU)
Sect. <i>Echenais</i>	
<i>C. echinus</i> (M. Bieb.) Hand.-Mazz.	Azerbaijan: Marand, Yam village, Mishodagh, 2200 m, Nouroozi 8600185 (HSBU)
Sect. <i>Cirsium</i>	
<i>C. obvallatum</i> (M. Bieb.) M. Bieb.	Azerbaijan: Meshkinshahr, Lahrood, Shabil, 2850 m, Nouroozi 8600175 (HSBU)
<i>C. libanoticum</i> DC.	Azerbaijan: Marand, Yam village, Mishodagh, 1900 m, Nouroozi 8600190 (HSBU)
<i>C. cereticum</i> (Lam.) d'Urv	Azerbaijan: Road of zabol to zahedan, Khaje mountains, 1300 m, Ghahreman (TUH)
<i>C. alatum</i> (S. G. Gmelin) Bobrov	Azerbaijan: Jolfa, road of Kaimakidagh, 736 m, Nouroozi 8600150 (HSBU)
<i>C. elodes</i> M. Bieb.	Azerbaijan: 35 km from Kaleybar to Ahar, 1700 m, Ghahreman & Mozzaffarian 17534 (TUH)
<i>C. canum</i> (L.) All.	Azerbaijan: Khoy, 1500 m, Nouroozi 8600120 (HSBU)
<i>C. hyrophilum</i> Boiss.	Tehran: Road of Haraz, lasem village, 1900 m, Nouroozi & Fathollahi 8600135 (HSBU)
<i>C. rhizocephalum</i> C. A. Mey.	Azerbaijan: Meshkinshahr, Lahrood, Shabil, 2850 m, Ghahreman & Mozzafarian 70121 (TUH)
Sect. <i>Cephalonoplos</i>	
<i>C. arvense</i> (L.) Scop.	Azerbaijan: Bostanabad, 1600 m, Nouroozi 8600120 (HSBU)

(Rechinger 1979) and have been classified into five sections and several subsections and series in the Flora. The genus is considered to be taxonomically complex due to the variability and intergradations of diagnostic characters among taxa. The complex nature of the genus may be partly due to a high degree of hybridization occurring among different species and also due to highly variable chromosome numbers seemingly interacting with different environmental conditions. Several hybrids have been described from the Caucasus and adjoining regions of Asia Minor (Ownbey 1951, Moore

and Frankton 1962, Charadze 1998, Bures et al. 2004).

Literature available on *Asteraceae* pollen is comprehensive, but publications have been very limited in Iran (Jafari and Ghanbarian 2007). Therefore, the present study analysed pollen morphology in 23 *Cirsium* species growing in Iran for the first time.

Materials and Methods

Pollen morphology was studied by light microscopy (LM) and scanning electron microscopy (SEM) in 23 *Cirsium* species,

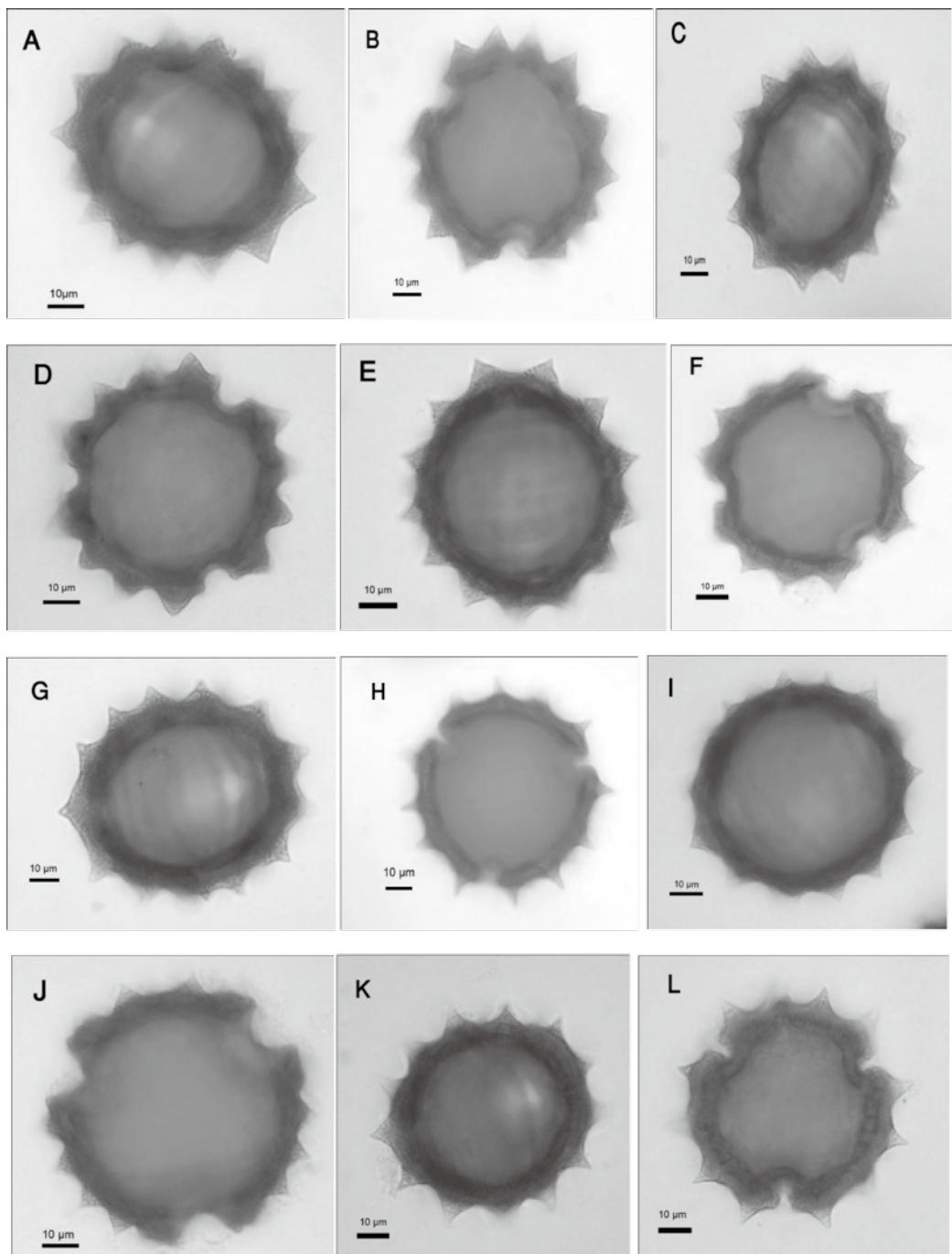


Fig. 1. Representative LM micrographs of pollen grains in *Cirsium* species studied. A. *C. spectabile* (equatorial view). B. *C. spectabile* (polar view). C. *C. pyramidale* (equatorial view). D. *C. pyramidale* (polar view). E. *C. congestum* (polar view). F. *C. congestum* (equatorial view). G. *C. bracteosum* (equatorial view). H. *C. bracteosum* (polar view). I. *C. burnmeulleri* (equatorial view). J. *C. burnmeulleri* (polar view). K. *C. strigosum* (equatorial view). L. *C. strigosum* (polar view). Scale bar = 10 μm .

representing five sections, growing in Iran (Table 1). The voucher specimens have been deposited in the Shahid Beheshti University Herbarium (HSBU) and Central Herbarium of Tehran University (TUH).

The pollen samples were obtained mostly from freshly collected specimens and also from dry herbarium materials. Fully matured anthers were removed from the specimens and prepared by the standard acetolysis method (Ernstman 1952, 1969), and then they were mounted in glycerin jelly and sealed with paraffin wax for light microscopy. Morphological studies were performed on minimum 20 pollen grains for each species by an Olympus light microscope Model BH-2.

For scanning electron microscopy, the acetolysed pollen grains were attached to aluminum stubs with double sided cellophane tape, air-dried at room temperature and coated with gold. The specimens were examined with a Philips XL 30 or a Leica LEO 440i at 15 kV and 22 kV and photographed.

UTHSCSA Image Tool ver. 3 software was used for pollen measurements, and then data obtained were scored. For multivariate analyses the mean of quantitative characters were used, while qualitative characters were coded as binary/multistate characters. Standardized data (mean = 0, variance = 1) were used for multivariate statistical analyses. The average taxonomic distance and squared Euclidean distance were used as dissimilarity coefficient in cluster analysis of data (Podani 2000).

Palynological characteristics studied included, polar axis, equatorial axis, ratio of polar axis/equatorial axis (P/E), spine length, exine thickness, spine base width, number of spine row between colpi, mesocolpium length, mean distance between two spines, number of spines per $100 \mu\text{m}^2$, pollen shape, exine ornamentation and spine tip shape. The terminology is according to Punt et al. (2007).

Analysis of variance (ANOVA) was performed among different sections studied for

quantitative palynological characters noticed, to show if these data may be used in section delimitation. Principal component analysis (PCA) was performed among the species studied to determine palynological characteristics useful for separating the species. In order to group the species, cluster analysis using UPGMA (Unweighted Paired Group with Arithmetic Average) and NJ (Neighbor-joining) methods and PCA ordination plot were performed using euclidean and taxonomic distances calculated among the species (Podani 2000). ANOVA and least significant differences (LSD) tests were conducted using SPSS ver. 9 (1998), while clustering and ordination plot analyses using NTSYS ver. 2 (1998).

Results

Results of palynological study are presented in Table 2 and Figures 1–5. All species studied, showed trizonocolporate (Fig. 1), isopolar and radially symmetrical pollen grains; with echinate sculpturing. Pollen outline is suboblate or oblate-spheroidal in equatorial view and circular to semi angular in polar view. The mean of polar axis ranges from 61.93 μm in *C. arvense* (Fig. 2D) to 79.85 μm in *C. osseticum*; the equatorial axis ranges from 68.20 μm in *C. aduncum* (Fig. 2A) to 87.99 μm in *C. obvallatum*. The mean width of mesocolpium ranges from 45.41 μm in *C. alatum* (Fig. 2B) to 71.75 μm in *C. libanoticum* (Fig. 2L). The exine ornamentation is echinate with microperforated tectum in most of the species studied (Fig. 3B–F, H) and with microgranulated in *C. aduncum* (Fig. 3A) and *C. haussknechtii* or microreticulated tectum in *C. alatum* (Fig. 3G), *C. cereticum* and *C. obvallatum*. The spines are conical with broad base and rounded (Fig. 3A–D) or rounded tip (Fig. 3E–H). The number of spine rows between two colpi varies between 4–5, resulting into about 12 to 15 spines in the equatorial plane. The distance between two spines ranges from 15.89 μm in *C. aduncum* (Fig. 2A) to 24.03 μm in *C. cereticum*. The mean number of spines per 100

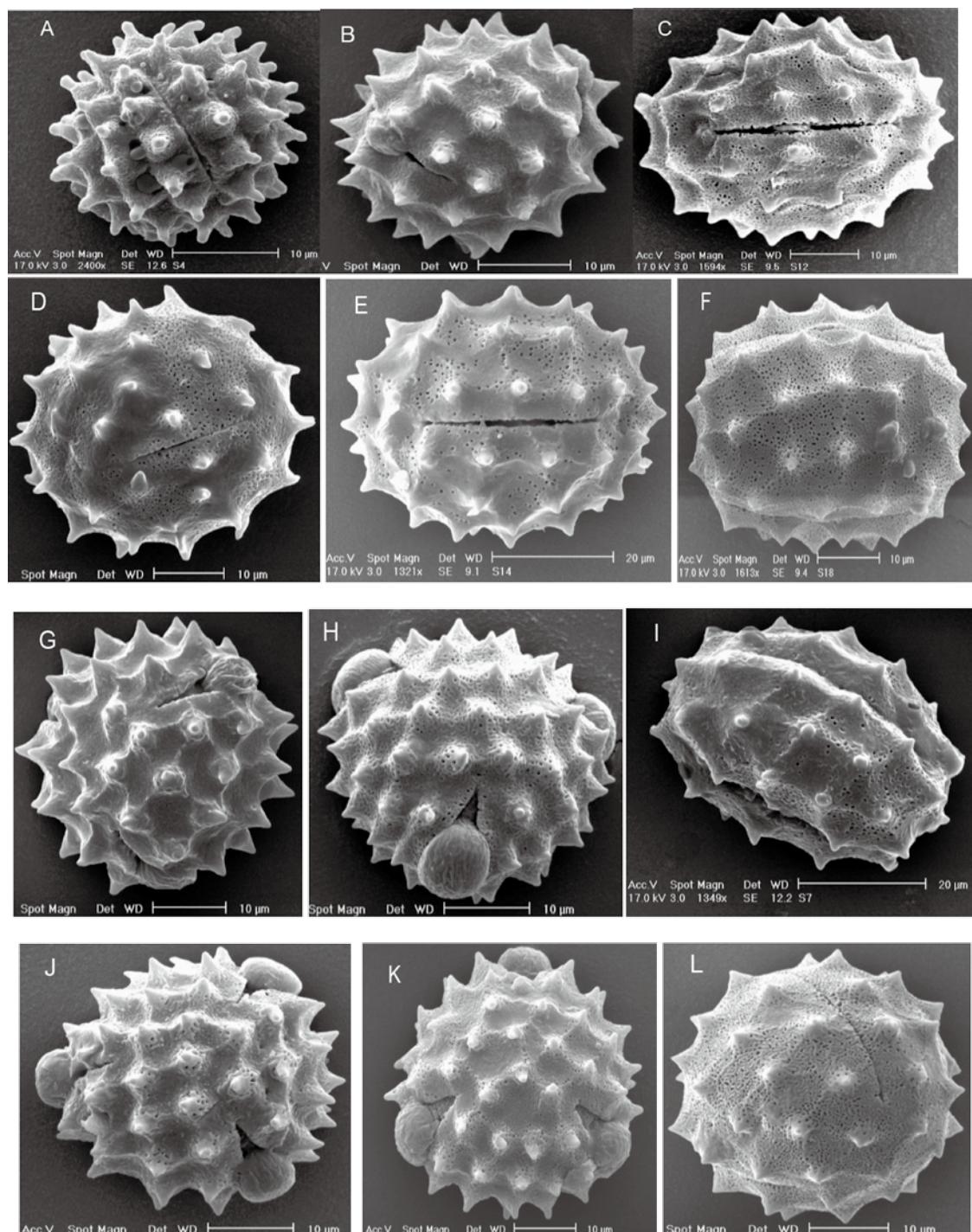


Fig. 2. Representative SEM micrograph of pollen grains in *Cirsium* species studied. A. *C. aduncum*. B. *C. alatum*. C. *C. bracteosum*. D. *C. arvense*. E. *C. spectabile*. F. *C. burnmeulleri*. G. *C. ciliatum*. H. *C. rhizocephalum*. I. *C. pyramidale*. J. *C. lappaceum*. K. *C. hygrophilum*. L. *C. libanoticum*.

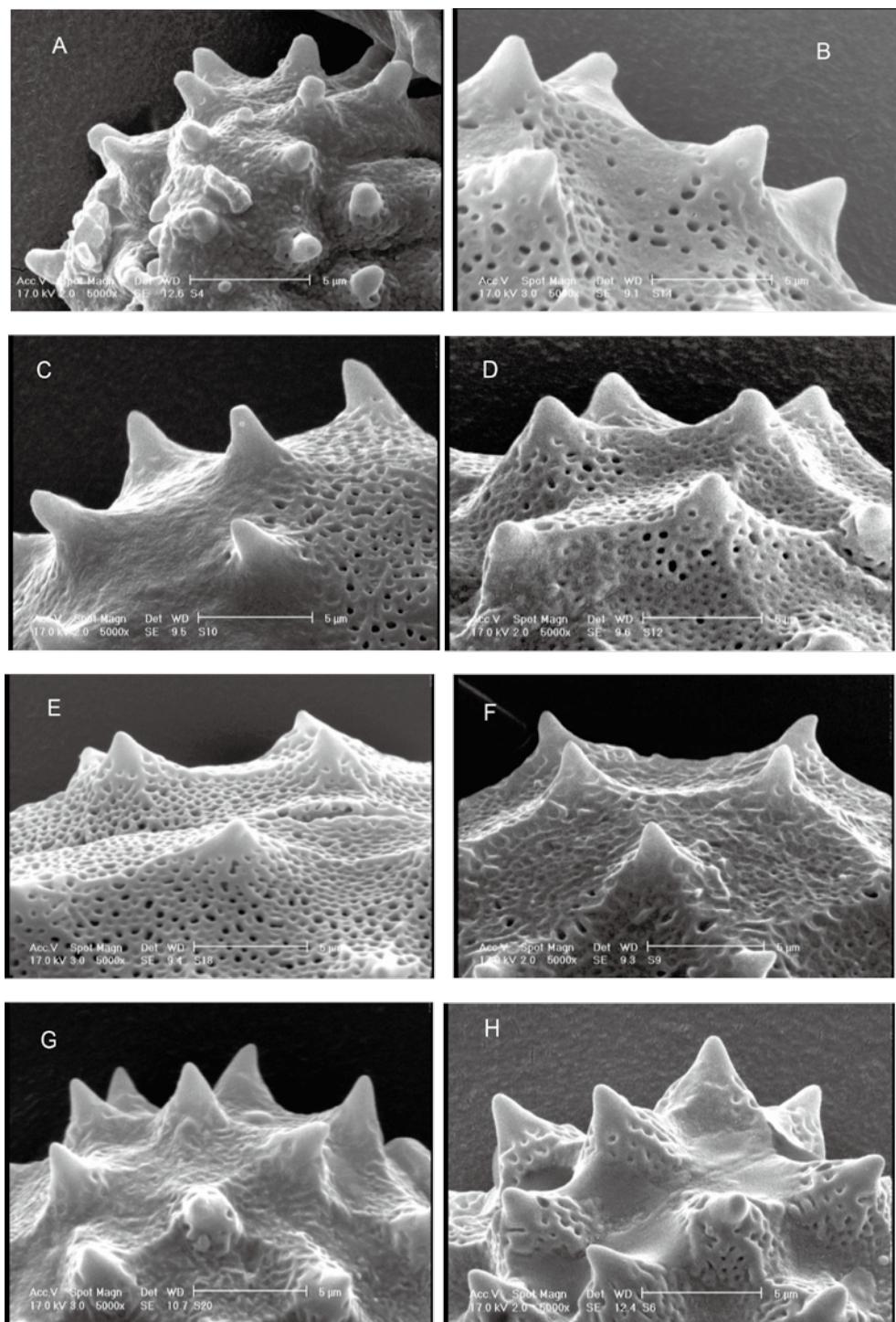


Fig. 3. Representative exine ornamentation in *Cirsium* species studied. A. *C. aduncum*. B. *C. spectabile*. C. *C. arvense*. D. *C. bracteosum*. E. *C. burnmeulleri*. F. *C. pyramidale*. G. *C. alatum*. H. *C. osseticum*.

Table 2. Pollen morphological parameters in *Cirsium* species examined. Data of statistical columns consists of the mean ± standard deviation in top line and rang in bottom line ximumon bottom line .

	Polar axis	Equatorial axis	P/E	Spine length	Eocene thickness	Spine base length	Number of spine rows	Pollen shape	Mesocolpium length	Distance of two spines	Number of spines per 100 μm ²	Ornamentation/spine tip
Sect. <i>Pseudopinacothecys</i>												
<i>C. spectabile</i>	74.91 ± 3.69	85.54 ± 2.81	0.87	10.00 ± 0.94	11.28 ± 0.70	15.97 ± 1.38	4	suboblate	64.07 ± 1.79	22.41 ± 1.49	3	microperforate/rounded
	70.22–80.45	80.26–90.55		8.44–11.46	10.12–12.36	13.89–17.86			61.99–65.14	20.11–24.22		
<i>C. congestum</i>	70.83 ± 2.34	85.34 ± 3.89	0.83	7.89 ± 0.55	9.87 ± 1.27	12.46 ± 0.84	4	suboblate	64.11 ± 2.19	21.63 ± 3.44	6	microperforate/pointed
	67.01–73.73	80.08–94.77		7.11–8.63	7.61–12.08	10.99–13.90			61.61–69.39	17.42–30.71		
<i>C. pyramidalis</i>	72.37 ± 5.13	86.66 ± 5.27	0.83	8.85 ± 0.44	10.86 ± 0.82	14.99 ± 1.16	4	suboblate	64.55 ± 2.43	22.91 ± 1.85	6	Microperforate/rounded
	65.44–79.88	78.35–95.02		7.99–9.42	9.85–12.24	12.80–16.93			60.94–67.58	20.83–26.42		
Sect. <i>Epitrichys</i>												
<i>C. bracteosum</i>	68.20 ± 2.57	76.85 ± 3.00	0.89	8.09 ± 0.71	9.87 ± 0.70	13.23 ± 0.86	4	Oblate-Spheroidal	59.04 ± 0.77	19.25 ± 1.32	4	microperforate/rounded
	64.39–72.01	70.30–82.13		6.80–9.62	8.61–11.34	11.60–14.98			57.99–59.67	17.43–22.59		
<i>C. burnetii</i>	69.83 ± 3.41	86.68 ± 3.90	0.91	7.14 ± 0.51	8.86 ± 0.56	13.59 ± 1.21	4	Oblate-Spheroidal	60.34 ± 4.75	19.10 ± 1.15	3	microperforate/pointed
	64.78–77.82	69.11–83.88		6.22–8.36	7.56–9.66	11.42–16.98			54.86–63.28	17.16–21.83		
<i>C. strigosum</i>	77.14 ± 3.96	83.29 ± 3.27	0.92	7.84 ± 1.05	9.92 ± 0.96	13.37 ± 2.02	4–5	Oblate-Spheroidal	66.82 ± 2.19	20.80 ± 1.67	5	microperforate/pointed
	72.83–83.82	78.45–87.15		6.76–10.13	8.22–11.09	11.03–18.10			64.40–68.69	18.80–23.24		
<i>C. ciliatum</i>	77.05 ± 4.30	87.25 ± 4.11	0.89	8.20 ± 1.00	10.19 ± 1.59	13.14 ± 1.23	4–5	Oblate-Spheroidal	64.20 ± 1.98	20.14 ± 2.37	4	microperforate/pointed
	70.83–85.52	80.99–92.62		6.60–9.86	7.28–12.32	11.50–15.04			62.09–66.55	15.77–23.93		
<i>C. osseum</i>	79.85 ± 5.58	84.55 ± 5.03	0.94	8.01 ± 0.59	9.48 ± 1.16	13.06 ± 0.72	4	Oblate-Spheroidal	71.12 ± 0.32	21.92 ± 1.84	5	microperforate/pointed
	71.54–87.40	76.20–90.43		7.28–8.98	7.30–10.71	11.80–14.04			70.89–71.35	19.67–25.25		
<i>C. aduncum</i>	64.61 ± 5.61	68.20 ± 5.88	0.95	6.50 ± 1.24	8.88 ± 0.66	9.48 ± 1.60	4–5	Oblate-Spheroidal	53.59 ± 3.86	15.89 ± 1.56	11.5	microgranulated/rounded
	56.50–72.42	57.40–77.47		5.00–8.41	7.95–10.05	6.60–12.08			47.22–56.64	13.17–18.09		
<i>C. haussknechii</i>	68.57 ± 6.01	73.33 ± 6.55	0.93	7.94 ± 0.56	11.30 ± 1.36	12.69 ± 1.08	4–5	Oblate-Spheroidal	56.14 ± 4.48	19.60 ± 1.96	5	microgranulated/rounded
	62.07–77.13	65.52–82.99		7.13–9.14	9.65–13.58	11.53–14.88			52.97–59.31	16.73–21.47		
<i>C. lappaceum</i>	71.19 ± 2.97	83.11 ± 3.41	0.89	9.51 ± 0.59	10.54 ± 0.94	15.39 ± 1.41	4	Oblate-Spheroidal	63.74 ± 0.86	22.09 ± 1.12	8	microperforate/pointed
	65.59–74.97	78.18–88.67		8.85–10.77	9.09–12.11	12.70–16.94			63.13–64.35	20.76–23.41		
<i>C. turkestanicum</i>	72.02 ± 2.41	81.79 ± 1.98	0.89	8.29 ± 0.65	10.05 ± 0.56	13.25 ± 0.93	4	Oblate-Spheroidal	63.76 ± 1.41	22.21 ± 1.08	4	microperforate/rounded
	68.34–75.91	79.90–83.88		7.40–9.48	9.25–11.32	11.55–14.53			62.12–65.16	21.07–23.92		

Table 2. Continued .

	Polar axis	Equatorial axis	P/E	Spine length	Exine thickness	Spine base length	Number of spine rows	Pollen shape	Mesocolpium length	Distance of two spines	Number of spines per 100 μm^2	Ornamentation/spine tip
<i>C. vulgare</i>	69.58±2.09 66.56–72.65	79.23±2.76 72.44–81.86	0.89 6.23–8.44	7.28±0.70 8.77–10.46	9.63±0.57 9.82–13.48	12.01±1.23 9.82–13.48	4–5	Oblate-Spheroidal	60.39±0.86 59.47–61.18	19.34±1.05 17.99–20.97	3	microporcate/rounded
Sect. <i>Cirsium</i>												
<i>C. obvallatum</i>	76.11±2.58 72.18–79.42	87.99±2.71 84.71–92.87	0.89 6.19–9.29	7.83±1.05 9.52–13.26	11.59±0.93 10.46–13.99	12.39±1.29 14.50±1.24	4–5	Oblate-Spheroidal	67.25±2.69 63.26–69.05	20.54±1.16 19.02–22.97	5	micreticulated/pointed
<i>C. libanoticum</i>	76.93±1.77 74.03–79.00	87.12±2.77 83.16–91.29	0.89 6.44–8.76	7.68±0.54 8.69–12.87	11.03±1.26 12.17–15.82	14.50±1.24 13.48±1.05	4–5	Oblate-Spheroidal	71.75±0.31 71.53–71.97	21.59±1.42 18.74–23.77	2	microporcate/pointed
<i>C. ceriticum</i>	71.67±1.84 68.07–74.95	83.19±3.02 76.23–87.41	0.89 7.83–9.80	8.93±0.63 8.46–11.89	10.01±0.97 11.80–15.23	13.48±1.05 11.80–15.23	4	Oblate-Spheroidal	66.34±1.27 64.54–67.62	24.03±1.61 20.73–26.43	2.5	micreticulated/pointed
<i>C. alatum</i>	68.60±2.84 64.97–73.57	76.57±3.85 70.62–82.33	0.89 6.56–8.80	7.34±0.66 8.07–9.81	9.24±0.56 10.07–12.46	11.11±0.77 10.07–12.46	4	Oblate-Spheroidal	45.41±3.76 40.00–48.32	19.88±1.90 17.25–23.49	7	micreticulated/pointed
<i>C. elodes</i>	63.38±2.38 58.04–65.62	69.49±3.07 63.78–72.52	0.91 6.49–8.16	7.44±0.61 8.65–11.48	9.91±0.95 11.44–15.57	13.09±1.32 11.13–14.45	4	Oblate-Spheroidal	51.51±1.24 50.64–52.39	18.87±1.75 16.35–21.56	6	microporcate/pointed
<i>C. canum</i>	70.95±2.90 66.07–76.28	80.95±4.04 72.37–84.83	0.89 7.45–10.19	8.87±0.93 7.88–9.94	8.89±0.65 13.95–18.81	16.13±1.45 12.71±1.38	5	Oblate-Spheroidal	59.88±2.64 56.00–63.44	20.83±1.60 18.55–23.98	3	microporcate/pointed
<i>C. hypoleophilum</i>	74.79±2.40 71.36–79.40	77.05±2.13 74.06–80.89	0.97 7.13–8.97	8.03±0.55 9.36–11.95	10.91±0.81 10.41–14.32	12.71±1.38 10.52–13.49	4–5	Oblate-Spheroidal	61.99±0.13 61.90–62.08	19.16±1.54 17.50–21.11	6	microporcate/pointed
<i>C. rhizocephalum</i>	64.54±2.44 61.21–68.29	73.07±2.86 68.50–78.61	0.88 5.79–8.06	6.77±0.66 8.66–11.52	10.08±0.97 10.52–13.49	11.70±0.93 10.52–13.49	5	Oblate-Spheroidal	57.68±2.05 55.20–59.85	17.28±1.09 15.40–18.85	7	microporcate/pointed
Sect. <i>Echenais</i>												
<i>C. Echinus</i>	69.82±3.20 67.17–78.29	76.50±2.76 72.91–83.01	0.91 6.45–9.24	7.49±0.79 8.60–10.69	9.72±0.64 11.17–13.86	12.19±0.84 10.41±1.39	5	Oblate-Spheroidal	59.59±1.70 57.02–63.67	17.24±1.04 15.40–19.24	5	microporcate/rounded
Sect. <i>Cephalonophos</i>												
<i>C. arvense</i>	61.93±2.52 59.14–67.37	73.25±3.28 67.67–69.02	0.85 5.72–8.64	7.06±0.98 7.77–9.62	8.56±0.62 7.97–13.44	10.41±1.39 9.77–13.44	4–5	suboblate	59.33±1.43 57.71–61.81	19.08±1.99 15.88–22.27	3	microporcate/rounded

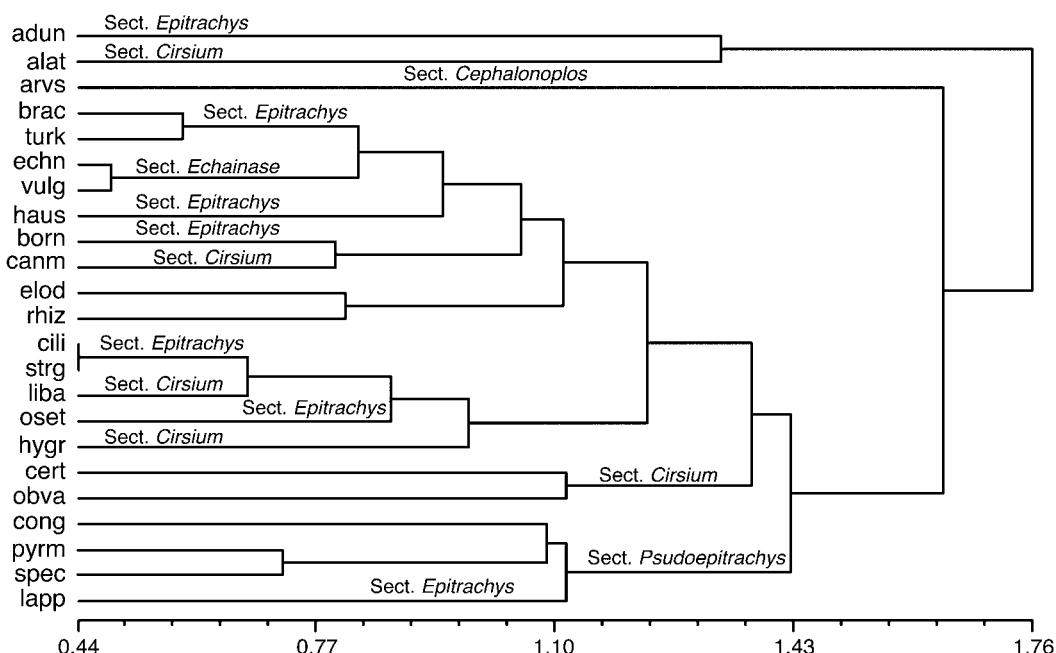


Fig. 4. UPGMA dendrogram of palynological data in *Cirsium* species studied.

μm^2 varies from 2 in *C. libanoticum* (Fig. 2L) to 11 in *C. aduncum* (Fig. 2A). The mean of spine length varies from 6.50 μm in *C. aduncum* (Figs. 2A, 3A) to 10.00 μm in *C. spectabile* (Figs. 2E, 3B). The mean width of spine base varies from 9.48 μm in *C. aduncum* (Fig. 3A) to 16.13 μm in *C. canum*. The mean of exine thickness ranges from 8.56 μm in *C. arvense* to 11.59 μm in *C. obvallatum*.

ANOVA test performed for pollen characteristics among five sections of Iranian *Cirsium* studied showed significant difference only for equatorial axis length ($F = 3.14$, $p < 0.05$). However, PCA analysis performed on these characters revealed that the first 3 components comprise about 65% of the total variation and in the first component (38% of variation), characters like equatorial axis length, polar axis length, echinae length, distance between two echinae, echinae base width and distance between two colpi have the highest positive correlation ($r > 0.70$).

UPGMA, NJ and WARD methods of clustering of pollen data produced similar results, with a higher cophenetic correlation ($r = 0.95$), which was also supported with respect to some species by ordination plot obtained (Figs. 4, 5).

Considering the species affinity represented in the UPGMA dendrogram (Fig. 4), in sect. *Psuedoepitrachys* *C. congestum*, *C. pyramidale* and *C. spectabile* formed a subcluster, which shows close affinity to each other. In sect. *Epitrachys*, *C. bracteosum* and *C. turkestanicum* were placed close to each other. *Cirsium haussknechtii* then joined these species with some distance. *Cirsium ciliatum*, on the other hand, showed affinity with *C. strigosum*, and *C. osseticum* joining these two species at some distance. However, *C. aduncum* and *C. lappaceum* were placed in different subclusters, respectively. In sect. *Cirsium*, *C. elodes* and *C. rhizocephalum* formed a subcluster showing some degree of similarity, and *C. canum* joined

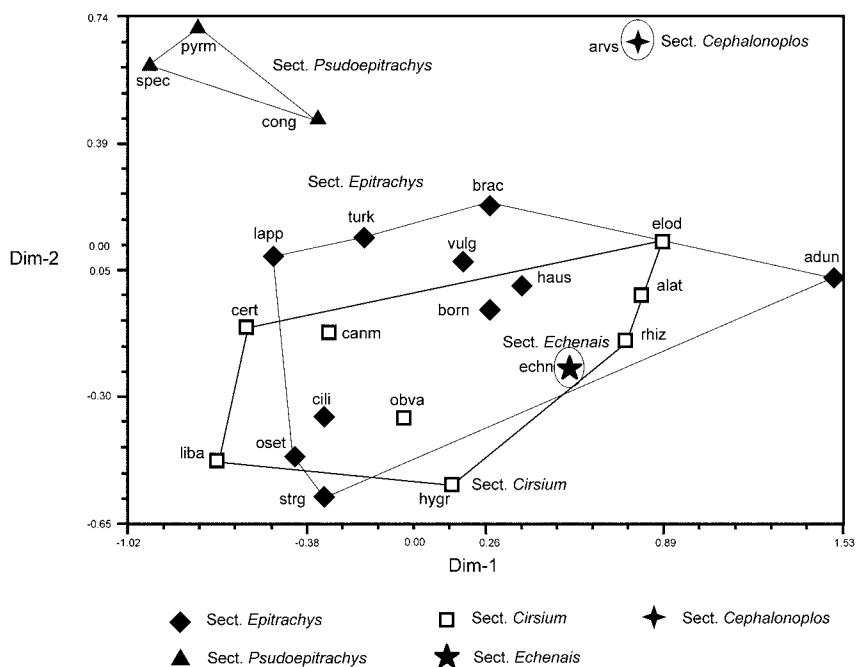


Fig. 5. PCA plot of palynological data in *Cirsium* species studied.

these species with some distance. *Cirsium libanicum* and *C. hygrophilum* as well as *C. cereticum* and *C. obvallatum*, however, showed affinity to each other but they were placed in different subclusters. *Cirsium alatum* was placed in the most separated subcluster with *C. aduncum* of the sect. *Epitrachys*.

PCA analysis showed that the first three components (coordinates) comprise about 65% of total variance in which the first, second and third components explained about 38%, 15% and 12% of the total variation, respectively. The ordination plot on first two component axes showed that plots of the sect. *Pseudoeprachys* and the sect. *Cephalonoplos* are separated from those of other sections along Dim-2 (Fig. 5).

Discussion

The species investigated in the present study are from all sections of Iranian *Cirsium*. Pollen grains observed are usually radially symmetrical, isopolar, trizonocolporate, oblate spheroidal

to spheroidal with echinate sculpturing, which is almost in agreement with previous reports on the genus *Cirsium*, although the shape of pollen grains was slightly differently described as suboblate or oblate-spheroidal (Halbritter et al. 2005), spheroidal to oblate-spheroidal or spheroidal to prolate-spheroidal (Meo 2005), spheroidal (Hesse et al. 2009), spherical (Jafari and Ghanbarian 2007), subprolate or prolate spheroidal (Yıldız et al. 2011) in equatorial view and is circular (Yıldız et al. 2011) or semiangular or semiangular to circular in polar view (Meo 2005).

According to Flora Iranica (Rechinger 1979) the genus *Cirsium* has five sections including *Cirsium*, *Cephalonoplos*, *Echenais*, *Epitrachys*, and *Pseudoeprachys*. In the present study, the species attributed to sectt. *Pseudoeprachys* and *Cephalonoplos* are all suboblate, while those attributed to sections *Epitrachys* and *Cirsium* are oblate-spheroidal. All the species have echinate sculpturing, and the exine ornamentation

between spines is microperforated in all species of sect. *Pseudoepitrachys*, *Echenais* and *Cephalonoplos*. The species of sect. *Epitrachys* have also microperforated exine excluding *C. aduncum* and *C. haussknechtii* that have microgranulated exine. *Cirsium aduncum* and *C. haussknechtii* are separated and attributed to subsect. *Microcephala* in the sect. *Epitrachys* due to the presence of small capitula (Rechinger 1979). The species of sect. *Cirsium* have microperforated exine excluding *C. cereticum*, *C. alatum* and *C. obvallatum* that have microreticulated exine.

The ANOVA result indicated that five sections of Iranian *Cirsium* do not differ significantly in their pollen characteristics and such data may not be used in discriminating different sections. However, the PCA result which represented the first three factors comprising about 65% of all of the variation, showed that characteristics such as polar axis length, equatorial axis length, echine length, distance between two colpi, distance between two echinae and the echine base length are the most variable pollen characteristics in the first factor with about 38.41% of the total variation and these characteristics may be used for species delimitation.

Clustering and ordination plot of the species based on pollen data also indicated that almost sectional delimitation is not possible by such characteristics because the species from different sections have been placed close to each other and are intermingled. However, in some places good separation has occurred, for example the species of *C. congestum*, *C. pyramidale* and *C. spectabile* from the sect. *Pseudepitrachys* almost form a separate group in the ordination plot (Figs. 4, 5). Moreover we find that in the sect. *Epitrachys*, *C. strigosum*, *C. ciliatum* and *C. osseticum* show close affinity supporting the treatment in *Flora Iranica* with respect to these species (Rechinger 1979). In sect. *Cirsium* the affinity of *C. cereticum* and *C. obvallatum* and

that of *C. elodes* and *C. rhizocephalum*, both suggested in *Flora Iranica* (Rechinger 1979), were also supported by their similarity in some degree from pollen characteristics.

Our previous cytotaxonomic study on Iranian *Cirsium* species (Nouroozi et al. 2010) revealed the affinity between *C. congesum* and *C. spectabile* from sect. *Pseudepitrachys* and that among *C. alatum*, *C. hygrophilum*, *C. libanoticum* and *C. obvallatum* from sect. *Cirsium*. Both affinities are almost supported by the present palynological data.

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M. Nouroozi^a, M. Sheidai^a, F. Attar^b, Z. Noormohammadi^c : イラン産アザミ属（キク科）の花粉形態学的研究

イラン産アザミ属（キク科）の5節（Sect. *Cirsium*, Sect. *Cephalonoplosn* (Necker) DC., Sect. *Echenais* (Cass.) Pettrak, Sect. *Epitrachys* DC., Sect. *Pseudepitrachys* Pettrak）に所属する23種の花粉形態を光学顕微鏡と走査型電子顕微鏡を用いて調べた。上記の23種のうち5種がイラン固有で、花粉データはこれまでに報告されていない。

花粉は放射相称、等極性 isopolar, 三帯状溝型 trizonocolporate, 表面は長刺型 echinate であった。テクタム（外表膜）は微散孔型 microperforate～微網状紋型 microreticulate, あるいは微粒状型 microgranulate であった。形状は赤道観は亜扁平型 suboblate～扁球型 oblate-spheroidal, 極観は円形～三角形であった。突

起は円錐形で、基部は広がり、先端は尖頂あるいは円頂。溝間の突起の列数はどの種でも4～5であった。ANOVA検定の結果、赤道軸長以外には有意な差は見られず、花粉のデータは節の区分には有用でないことが分かった。主成分分析では赤道軸長と二つの溝間の距離、及び極軸長が種の区別に重要であることが分かった。また、*Pseudepitrachys* 節の種にまとまりがあることと、*Cephalonoplos* 節が他の節とは異なることを示したが、この他の節が花粉の形態にもとづいたまとまりを示すことはなかった。

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